

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	23017	chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L2	4651	lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L3	137806	promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L4	16883	"antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L5	649	lox SAME promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L6	5764	"antibiotic resistance gene"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L7	2	"5527695".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L8	18	(lox SAME promoter) SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L9	59	lox SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L10	4	"6143557".pn. or "5888732".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L11	22	chloramphenicol SAME lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L12	159	"antibiotic resistance gene" SAME chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45

L13	27	("antibiotic resistance gene" SAME chloramphenicol) AND "site specific recombination"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L14	19	chloramphenicol SAME "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L15	78	chloramphenicol AND "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L16	14527	promoter and "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L17	90863	"immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L18	251	promoter and "antibiotic resistance" and (lox SAME promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L19	27	(promoter and "antibiotic resistance" and (lox SAME promoter)) and "immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L20	23017	chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L21	4651	lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L22	137806	promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L23	16883	"antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L24	649	lox SAME promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45

L25	5764	"antibiotic resistance gene"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L26	14527	promoter and "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L27	90863	"immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L28	251	promoter and "antibiotic resistance" and (lox SAME promoter)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L29	2	"5527695".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L30	18	(lox SAME promoter) SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L31	59	lox SAME "antibiotic resistance"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L32	4	"6143557".pn. or "5888732".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L33	22	chloramphenicol SAME lox	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L34	27	("antibiotic resistance gene" SAME chloramphenicol) AND "site specific recombination"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L35	19	chloramphenicol SAME "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L36	78	chloramphenicol AND "bacterial selection"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45

L37	27	(promoter and "antibiotic resistance" and (lox SAME promoter)) and "immediately adjacent"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L38	159	"antibiotic resistance gene" SAME chloramphenicol	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:45
L39	305	lambdoid	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:49
L40	2	I39 SAME (att near3 site)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:53
L41	138	(att near3 site) and I3 and I4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:53
L42	22	I41 and I39	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:56
L43	3474	hartley.in. or brasch.in. or invitrogen\$.as.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:57
L44	51	hartley.in. and brasch.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:56
L45	6	I44 and lambdoid	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 15:56
L46	26	I43 and I41	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:08
L47	3	"10162879"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:08
L48	0	I47 and lambdoid	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:08

L49	5	"10058292"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:08
L50	0	I49 and Iambdoid	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:25
L51	43771	(I536/23.1 536/24.1 435/320.1 435/252.1 435/325 .ccs.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:28
L52	430	I51 and I1 and I2	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:28
L53	218	I52 and I4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:28
L54	25	I53 and I17	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 16:30
L55	2	"5677177".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:26
L56	7032	att and Iambda	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:27
L57	7073	promoter SAME I4	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:27
L58	1012	I57 and I56	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:27
L59	785	I58 and recombination	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:27
L60	182	I59 and (recombination near2 site)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/10/11 17:28

	Document ID	Title
1	US 20050124010 A1	Whole cell engineering by mutagenizing a substantial portion of a starting genome combining mutations and optionally repeating
2	US 20040092016 A1	Enhanced homologous recombination mediated by lambda recombination proteins
3	US 20040077090 A1	Whole cell engineering by mutagenizing a substantial portion of a starting genome, combining mutations, and optionally repeating
4	US 20040016025 A1	Rice promoters for regulation of plant expression
5	US 20030224521 A1	Enhanced homologous recombination mediated by lambda recombination proteins
6	US 20030148309 A1	Evolution of whole cells and organisms by recursive sequence recombination
7	US 20030124555 A1	Compositions and methods for use in isolation of nucleic acid molecules
8	US 20030104604 A1	Genetically engineered bacterial strains for the display of foreign peptides on filamentous phage
9	US 6828093 B1	Rapid subcloning using site-specific recombination

	Document ID	Title
10	US 6720140 B1	Recombinational cloning using engineered recombination sites
11	US 6716631 B1	Evolution of whole cells and organisms by recursive sequence recombination
12	US 6528311 B1	Evolution of whole cells and organisms by recursive sequence recombination
13	US 6379964 B1	Evolution of whole cells and organisms by recursive sequence recombination
14	US 6352859 B1	Evolution of whole cells and organisms by recursive sequence recombination
15	US 6326204 B1	Evolution of whole cells and organisms by recursive sequence recombination
16	US 6287862 B1	Evolution of whole cells and organisms by recursive sequence recombination
17	US 6277608 B1	Recombinational cloning using nucleic acids having recombination sites
18	US 6270969 B1	Recombinational cloning using engineered recombination sites
19	US 6251674 B1	Evolution of whole cells and organisms by recursive sequence recombination

	Document ID	Title
20	US 6171861 B1	Recombinational cloning using engineered recombination sites
21	US 6143557 A	Recombination cloning using engineered recombination sites
22	US 5888732 A	Recombinational cloning using engineered recombination sites

FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 17:40:40 ON 11 OCT 2005

L1 11576 S HARTLEY?/AU OR BRASCH?/AU OR INVITROGEN?
L2 85767 S LAMBDA OR LAMBDOID
L3 1128 S RECOMBINATION (2W) SITE
L4 19 S ATT AND L3
L5 9 S L4 AND L2
L6 4 DUP REM L5 (5 DUPLICATES REMOVED)
L7 53570 S EXPRESSION (2W) (VECTOR OR PLASMID OR CONSTRUCT OR NUCLEIC OR
L8 20 S L7 AND L3
L9 0 S L8 AND ATT
L10 57 S L7 AND ATT
L11 2 S L10 AND LAMBDA
L12 2 DUP REM L11 (0 DUPLICATES REMOVED)
L13 1037 S ATTB OR ATTP
L14 23 S L13 (S) ANTIBIOTIC
L15 40 S L13 (S) PROMOTER
L16 3 S L14 NOT PY>=1997
L17 1 DUP REM L16 (2 DUPLICATES REMOVED)
L18 26 S L15 NOT PY>=1999
L19 11 DUP REM L18 (15 DUPLICATES REMOVED)
L20 1 S L19 AND L7
L21 431 S ATT (P) (PROMOTER OR ANTIBIOTIC OR SELECTABLE OR SELECTION)
L22 253 S L21 NOT PY>=1997
L23 114 DUP REM L22 (139 DUPLICATES REMOVED)
L24 2 S L23 AND ANTIBIOTIC RESISTANCE
L25 4 S L23 AND L7
L26 47 S L1 AND L2
L27 4 S L26 AND L13
L28 2 DUP REM L27 (2 DUPLICATES REMOVED)
L29 0 S L1 AND L3 AND L13
L30 40 S L3 AND L13
L31 2 S L30 AND L7
L32 1 DUP REM L31 (1 DUPLICATE REMOVED)
L33 20 S ADJACENT (S) L21
L34 20 S ADJACENT AND L21
L35 8 DUP REM L34 (12 DUPLICATES REMOVED)
L36 7 S L35 NOT PY>=1999

=>

L36 ANSWER 1 OF 7 MEDLINE on STN
 ACCESSION NUMBER: 93224015 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 8468005
 TITLE: Expression of the Escherichia coli ftsZ gene: trials and tribulations of gene fusion studies.
 AUTHOR: Robin A; D'Ari R
 CORPORATE SOURCE: Institut Jacques Monod, C.N.R.S., Universite Paris, France.
 SOURCE: Genetical research, (1993 Feb) 61 (1) 1-8.
 Journal code: 0370741. ISSN: 0016-6723.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199305
 ENTRY DATE: Entered STN: 19930521
 Last Updated on STN: 19930521
 Entered Medline: 19930511

AB The ftsZ gene of Escherichia coli, which codes for an essential cell division protein, is subjected to multiple regulation, as shown in part with studies using an ftsZ::lacZ operon fusion located on phage lambda JFL100. Using this same fusion, we sought to isolate regulatory mutants overexpressing ftsZ by selecting mutants able to grow on lactose. One Lac+ mutant was obtained which overexpressed the ftsZ::lacZ fusion 70-fold. The mutation responsible for the overexpression lies in a new gene, cot, located near 56 min on the E. coli genetic map. The cot mutation probably affects the transcription of a chromosomal open reading frame, ORF1, lying downstream of the bioA gene and **adjacent** to the ftsZ::lacZ fusion of the lambda JFL100 prophage integrated at **att** lambda. Using an ftsZ84(Ts) strain, in which there was a double **selection** for overexpression of both ftsZ::lacZ and ftsZ+, no Lac+Tr mutants were obtained from 3.6 x 10(10) bacteria; the introduction of a mutL allele, increasing spontaneous base substitution mutation rates 75-fold, did not permit us to isolate such a mutant. We conclude that Lac+ ftsZ-constitutive mutations cannot be obtained in lambda JFL100 lysogens by a single base substitution.

L36 ANSWER 2 OF 7 MEDLINE on STN
 ACCESSION NUMBER: 93011141 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 1396706
 TITLE: Footprinting studies of DNA-sequence recognition by nogalamycin.
 AUTHOR: Fox K R; Alam Z
 CORPORATE SOURCE: Department of Physiology and Pharmacology, University of Southampton, England.
 SOURCE: European journal of biochemistry / FEBS, (1992 Oct 1) 209 (1) 31-6.
 Journal code: 0107600. ISSN: 0014-2956.
 PUB. COUNTRY: GERMANY: Germany, Federal Republic of
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199211
 ENTRY DATE: Entered STN: 19930122
 Last Updated on STN: 19930122
 Entered Medline: 19921113

AB We have studied the DNA sequence binding preference of the antitumour **antibiotic** nogalamycin by DNase-I footprinting using a variety of DNA fragments. The DNA fragments were obtained by cloning synthetic oligonucleotides into longer DNA fragments and were designed to contain isolated ligand-binding sites surrounded by repetitive sequences such as (A)n.(T)n and (AT)n. Within regions of (A)n.(T)n, clear footprints are observed with low concentrations of nogalamycin (< 5 microM), with apparent binding affinities for tetranucleotide sequences which decrease in the order TGCA > AGCT = ACGT > TCGA. In contrast, within regions of (AT)n, the ligand binds best to AGCT; binding to TCGA and TGCA is no stronger than to alternating AT. Within **(ATT)n**, the preference is for ACGT > TCGA. Although each of these binding sites contains all

four base pairs, there is no apparent consensus sequence, suggesting that the selectivity is affected by local DNA dynamic and structural effects. At higher drug concentrations (> 25 microM), nogalamycin prevents DNase-I cleavage of (AT)n but shows no interaction with regions of (AC)n.(GT)n. Regions of (A)n.(T)n, which are poorly cut by DNase I, show enhanced rates of cleavage in the presence of low concentrations of nogalamycin, but are protected from cleavage at higher concentrations. We suggest that this arises because drug binding to **adjacent** regions distorts the DNA to a structure which is more readily cut by the enzyme and which is better able to bind further ligand molecules.

L36 ANSWER 3 OF 7 MEDLINE on STN
ACCESSION NUMBER: 92194474 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1548767
TITLE: Both substrate and target oligonucleotide sequences affect in vitro integration mediated by human immunodeficiency virus type 1 integrase protein produced in *Saccharomyces cerevisiae*.
AUTHOR: Leavitt A D; Rose R B; Varmus H E
CORPORATE SOURCE: Department of Microbiology and Immunology, University of California, San Francisco 94143.
SOURCE: Journal of virology, (1992 Apr) 66 (4) 2359-68.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920509
Last Updated on STN: 19970203
Entered Medline: 19920417

AB Integration of retroviral DNA into the host cell genome requires the interaction of retroviral integrase (IN) protein with the outer ends of both viral long terminal repeats (LTRs) to remove two nucleotides from the 3' ends (3' processing) and to join the 3' ends to newly created 5' ends in target DNA (strand transfer). We have purified the IN protein of human immunodeficiency virus type 1 (HIV-1) after production in *Saccharomyces cerevisiae* and found it to have many of the properties described for retroviral IN proteins. The protein performs both 3' processing and strand transfer reactions by using HIV-1 or HIV-2 attachment (**att**) site oligonucleotides. A highly conserved CA dinucleotide **adjacent** to the 3' processing site of HIV-1 is important for both the 3' processing and strand transfer reactions; however, it is not sufficient for full IN activity, since alteration of nucleotide sequences internal to the HIV-1 U5 CA also impairs IN function, and Moloney murine leukemia virus **att** site oligonucleotides are poor substrates for HIV-1 IN. When HIV-1 **att** sequences are positioned internally in an LTR-LTR circle junction substrate, HIV-1 IN fails to cleave the substrate preferentially at positions coinciding with correct 3' processing, implying a requirement for positioning **att** sites near DNA ends. The 2 bp normally located beyond the 3' CA in linear DNA are not essential for in vitro integration, since mutant oligonucleotides with single-stranded 3' or 5' extensions or with no residues beyond the CA dinucleotide are efficiently used. **Selection** of target sites is nonrandom when **att** site oligonucleotides are joined to each other in vitro. We modified an in vitro assay to distinguish oligonucleotides serving as the substrate for 3' processing and as the target for strand transfer. The modified assay demonstrates that nonrandom usage of target sites is dependent on the target oligonucleotide sequence and independent of the oligonucleotide used as the substrate for 3' processing.

L36 ANSWER 4 OF 7 MEDLINE on STN
ACCESSION NUMBER: 91260671 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2046656
TITLE: Site-specific recombination in *Escherichia coli* between the att sites of plasmid pSE211 from *Saccharopolyspora erythraea*.

AUTHOR: Katz L; Brown D P; Donadio S
CORPORATE SOURCE: Corporate Molecular Biology, Abbott Laboratories, IL 60064.
SOURCE: Molecular & general genetics : MGG, (1991 May) 227 (1)
155-9.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910802
Last Updated on STN: 19970203
Entered Medline: 19910712

AB pSE211 from *Saccharopolyspora erythraea* integrates site-specifically into the chromosome through conservative recombination between attP and attB, the plasmid and chromosomal attachment sites. Integration depends on the presence of int, an open reading frame (ORF) that lies **adjacent** to attP and encodes the putative integrase. Immediately upstream of int lies xis (formerly called orf2) which encodes a basic protein that is thought to exhibit DNA binding. xis and int were cloned in various combinations in pUC18 and expressed constitutively in *Escherichia coli* from the lac **promoter**. attP and attB were cloned in *Streptomyces* or *E. coli* plasmids containing kanamycin resistance (KmR) or chloramphenicol resistance (CmR) markers. Stable KmR CmR cointegrates formed by attP x attB or attP x attP recombination (integration) were obtained in *E. coli* hosts that expressed int. Co-integrates were not found in hosts expressing int + xis. Excision (intraplasmid **att** site recombination) was examined by constructing plasmids carrying attL and attR or two attP sites separating CmR from KmR and by following segregation of the markers in various hosts. Both attL x attR and attP x attP excision depended on both xis and int in *E. coli*. pSE211 **att** site integration and excision were not affected by a deletion in himA, the gene encoding a subunit of integration host factor.

L36 ANSWER 5 OF 7 MEDLINE on STN
ACCESSION NUMBER: 87226168 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2438418
TITLE: *Escherichia coli* protein synthesis initiation factor IF3 controls its own gene expression at the translational level in vivo.
AUTHOR: Butler J S; Springer M; Dondon J; Graffe M; Grunberg-Manago M
SOURCE: Journal of molecular biology, (1986 Dec 20) 192 (4) 767-80.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198707
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19980206
Entered Medline: 19870723

AB Measurements of the relative synthesis rates of mRNAs transcribed from the gene (thrS) for threonyl-tRNA synthetase and the **adjacent** gene (infC) for initiation factor IF3 show four- to fivefold more infC mRNA than thrS mRNA in vivo, suggesting that infC expression can be controlled independently of thrS expression. S1 mapping experiments reveal the existence of two transcription initiation sites for infC mRNAs internal to the thrS structural gene. Both the mRNA measurements and the S1 mapping experiments indicate that the majority of infC transcription initiates at the infC proximal **promoter**. In agreement with these results, the deletion of the infC distal **promoter** from infC-lacZ gene fusions does not affect the expression of these gene fusions in vivo. Measurements of the relative synthesis rate of infC mRNA in vivo in infC-strains overproducing IF3 shows that infC mRNA levels are normal in these strains, thus suggesting that IF3 regulates the translation of infC mRNAs in vivo. Extension of these experiments using infC-lacZ gene fusions carried on lambda bacteriophage and integrated at the lambda **att**

site on the *Escherichia coli* chromosome shows that the expression of *infC-lacZ* protein fusions, but not *infC-lacZ* operon fusions, is derepressed in two *infC*- strains. A cellular excess of IF3 represses the expression of an *infC-lacZ* protein fusion but not an *infC-lacZ* operon fusion. Measurements of the relative mRNA synthesis rates of hybrid *infC-lacZ* mRNA synthesized from an *infC-lacZ* protein fusion under conditions of a fourfold derepression or a threefold repression of hybrid IF3-beta-galactosidase expression shows that the hybrid *infC-lacZ* mRNA levels remain unchanged. These results indicate that the cellular levels of IF3 negatively regulate the expression of its own gene, *infC*, at the translational level in vivo.

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ACCESSION NUMBER: 93140462 EMBASE
DOCUMENT NUMBER: 1993140462
TITLE: Identification of a putative *infC-rpmI-rplT* operon flanked by long inverted repeats in *Mycoplasma fermentans* (incognitus strain).
AUTHOR: Hu W.S.; Wang R.Y.-H.; Shih J.W.-K.; Lo S.-C.
CORPORATE SOURCE: Dept Infectious/Parasitic Dis Pathol, Armed Forces Institute of Pathology, Washington, DC 20306-6000, United States
SOURCE: Gene, (1993) Vol. 127, No. 1, pp. 79-85.
ISSN: 0378-1119 CODEN: GENED6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 021 Developmental Biology and Teratology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 930613
Last Updated on STN: 930613

AB A specific 1542-bp DNA fragment was amplified from *Mycoplasma fermentans* (incognitus strain) using a unique 23-nucleotide (nt) synthetic deoxyribonucleotide (oligo) (5'-TCCAAAAGTCCGGAATTGGGG) as the primer pair in the polymerase chain reaction (PCR). The 23-nt sequence is part of the 29-bp terminal inverted repeat (IR) which forms the left potential stem-and-loop (s and l) structure of the previously identified M. fermentans insertion-sequenced (IS)-like genetic element [Hu et al., Gene 93 (1990) 67-72]. The amplified DNA was cloned and sequenced. A pair of 27-bp IR containing the 23-nt synthetic oligo was identified at both termini. Between the IR, there are four potential open reading frames (ORFs) which are arranged **adjacent** to each other in the order, ORF-1, ORF-2, ORF-3 and ORF-4, with parts of ORF-1 and ORF-2 overlapping. The deduced amino acid (a) sequences of ORF-2, ORF-3, and ORF-4 are 34 to 60% identical to the translation initiation factor IF3 (encoded by the *infC* gene), ribosomal proteins L35 (*rpmI* gene) and L20 (*rplT* gene) of *Escherichia coli* and *Bacillus stearothermophilus*, respectively. In bacteria, the *infC-rpmI-rplT* genes are organized to function as an operon. There are multiple sites with **promoter**-like sequences identified upstream from the putative *infC* gene in the mycoplasma closely resembling the gene arrangement in the bacterial operon. All three genes of ORF-2, ORF-3 and ORF-4 are preceded individually by a strong appropriately spaced (7 and 10 bp) putative Shine-Dalgarno sequence (5'-AAGGA). In addition, ORF-2 uses the unusual triplet, **ATT**, as the codon, the same as that for *infC* in the bacterial operon. Thus, the cluster of genes (ORF-2, ORF-3 and ORF-4) is identified as a putative mycoplasma *infC-rpmI-rplT* operon. Most interestingly, our study reveals that this operon potentially constitutes a part of a mobile genetic element in the incognitus strain of M. fermentans.

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ACCESSION NUMBER: 80036670 EMBASE
DOCUMENT NUMBER: 1980036670
TITLE: Differential modes of processing and decay for the major N-dependent RNA transcript of coliphage.

AUTHOR: Wilder D.A.; Lozeron H.A.
CORPORATE SOURCE: Edward A. Doisy Dept. Biochem., St Louis Univ. Sch. Med.,
St Louis, Mo. 63104, United States
SOURCE: Virology, (1979) Vol. 99, No. 2, pp. 241-256.
CODEN: VIRLAX
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
004 Microbiology
LANGUAGE: English
ENTRY DATE: Entered STN: 911209
Last Updated on STN: 911209

AB The major leftward, N-dependent RNA synthesized from the pL (leftward **promoter**)-**att** operon of bacteriophage λ in wild-type and ribonuclease III (RNase III)-deficient hosts has been investigated by transcriptional mapping analysis. These studies reveal a sequence-specific program of post-transcriptional events leading to differential modes of RNA processing and decay of the N-dependent (13') RNA transcript. The initial events, which comprise the RNA processing (rapid) phase, include RNase II-mediated cleavage of the pL 11° (containing the 5'-ppp terminus) and the next **adjacent** 11 RNA species (both about 4.5 S in size) and a third cleavage event in the vicinity of the leftward transcriptional termination signal (tL1) just to the left of gene N. This latter cleavage appears to be responsible for the differentially rapid decay of the gene N RNA segment derived from the 3'-end of 111-tL1. This series of events is completed by the time the N-dependent transcription is terminated at the **att** site. The events of the succeeding secondary (relatively slow) phase was associated with the remaining tL1-**att**(13 RNA) segment and comprise the initiation of endonucleolytic cleavage and subsequent chemical decay events which result in the uniform decay of the entire 13 RNA segment of the 13' RNA transcript.

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L12 ANSWER 1 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:295591 BIOSIS
DOCUMENT NUMBER: PREV200100295591
TITLE: **Expression plasmid with a very tight**
two-step control: Int/**att**-mediated gene inversion
with respect to the stationary promoter.
AUTHOR(S): Sektas, Marian; Hasan, Noaman; Szybalski, Wacław [Reprint
author]
CORPORATE SOURCE: McArdle Laboratory for Cancer Research, Medical School,
Madison, WI, 53706-1599, USA
mariansektas@hotmail.com; nmhasan@facstaff.wisc.edu;
szybalski@oncology.wisc.edu
SOURCE: Gene (Amsterdam), (18 April, 2001) Vol. 267, No. 2, pp.
213-220. print.
CODEN: GENED6. ISSN: 0378-1119.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Jun 2001
Last Updated on STN: 19 Feb 2002

AB A very tightly controlled **expression vector** was
constructed, which was originally designed as to be able to use any
promoter, constitutive or regulated. Moreover, in vector pNH46T1, the
repressible Ptac/Plac promoters were used to transcribe genes cloned in
the proximal multiple cloning site (MCS), which was flanked by convergent
attB and attP sites. The gene of interest was cloned into MCS in the OFF
orientation, i.e. facing the promoter(s). In such OFF orientation, the
cloned gene could not be expressed, and only its anti-sense mRNA could be
produced. Four strong rrnBT1 terminators, in a tandem arrangement and
proximal to the N-terminal end of the cloned non-inverted gene, were
protecting it from any inadvertent transcription originating in the
vector. Moreover, the Ptac/Plac promoters/operators are controlled by the
LacIqts and LacI+ repressor(s) that further reduce the basal gene
expression in the uninduced state. When induced, the total vector
population is converted to the ON orientation by expression of the Int
function that inverts the attB and attP-flanked MCS including the cloned
gene. This places the gene under direct control of the Ptac/Plac
promoters, and thus results in very high expression. An additional
feature is the anti-termination system that consists of the
promoter-proximal nutL site and the inducible gene N, whose role in the ON
state is to overcome the rrnBT1 terminators and any other adventitiously
cloned terminators.

L12 ANSWER 2 OF 2 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights
reserved on STN
ACCESSION NUMBER: 1998000242 EMBASE
TITLE: A new DNA vehicle for nonviral gene delivery: Supercoiled
minicircle.
AUTHOR: Darquet A.-M.; Cameron B.; Wils P.; Scherman D.; Crouzet J.
CORPORATE SOURCE: J. Crouzet, Rhone-Poulenc Rorer Gencell, Centre Recherche
Vitry-Alfortville, 13 Quai Jules Guesdes, 94403 Vitry sur
Seine, France
SOURCE: Gene Therapy, (1997) Vol. 4, No. 12, pp. 1341-1349.
Refs: 35
ISSN: 0969-7128 CODEN: GETHEC
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19980120
Last Updated on STN: 19980120

AB Plasmids currently used for nonviral gene transfer have the disadvantage
of carrying a bacterial origin of replication and an antibiotic resistance
gene. There is, therefore, a risk of uncontrolled dissemination of the
therapeutic gene and the antibiotic resistance gene. Minicircles are new
DNA delivery vehicles which do not have such elements and are consequently

safer as they exhibit a high level of biological containment. They are obtained in *E. coli* by **att** site-specific recombination mediated by the phage **.lambda.** integrase. The desired eukaryotic expression cassette bounded by the **.lambda.** attP and attB sites was cloned on a recombinant plasmid. The expression cassette was excised in vivo after thermoinduction of the integrase gene leading to the formation of two supercoiled molecules: the minicircle and the starting plasmid lacking the expression cassette. In various cell lines, purified minicircles exhibited a two- to 10-fold higher luciferase reporter gene activity than the unrecombined plasmid. This could be due to either the removal of unnecessary plasmid sequences, which could affect gene expression, or the smaller size of minicircle which may confer better extracellular and intracellular bioavailability and result in improved gene delivery properties.

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L17 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 94341567 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8063103
TITLE: Conjugal transfer of cosmid DNA from Escherichia coli to
Saccharopolyspora spinosa: effects of chromosomal
insertions on macrolide A83543 production.
AUTHOR: Matsushima P; Broughton M C; Turner J R; Baltz R H
CORPORATE SOURCE: Lilly Research Laboratories, Eli Lilly and Company,
Indianapolis, IN 46285.
SOURCE: Gene, (1994 Aug 19) 146 (1) 39-45.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 19941005
Entered Medline: 19940921

AB Cosmid pOJ436, containing large inserts of Saccharopolyspora spinosa (Ss) DNA, was transferred by conjugation from Escherichia coli to Ss and integrated into the chromosome, apparently by homologous recombination, at high frequencies (10^{-5} to 10^{-4}) per recipient). Transfer was mediated by the plasmid RP4 (RK2) transfer functions in E. coli, and the RK2 oriT function located on pOJ436 [Bierman et al., Gene 116 (1992) 43-49]. pOJ436 lacking Ss DNA, or containing a small insert (approx. 2 kb) of Ss DNA, conjugated from E. coli and integrated at either of two bacteriophage phi C31 attB sites at low frequency (approx. 10^{-7}) per recipient). Exconjugants containing homologous inserts or inserts at the phi C31 attB sites were stable in the absence of antibiotic selection, and most produced control levels of tetracyclic macrolide A83543 factors. Some exconjugants contained similar kinds of large deletions and were defective in macrolide production.

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L20 ANSWER 1 OF 1 MEDLINE on STN
ACCESSION NUMBER: 88056310 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2960590
TITLE: Control of cloned gene expression by promoter inversion in vivo: construction of improved vectors with a multiple cloning site and the Ptac promoter.
AUTHOR: Hasan N; Szybalski W
CORPORATE SOURCE: McArdle Laboratory for Cancer Research, University of Wisconsin, Madison 53706.
CONTRACT NUMBER: 5-P30-CA-07175 (NCI)
5-PO1-CA-23076 (NCI)
SOURCE: Gene, (1987) 56 (1) 145-51.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198801
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19880119

AB We have constructed three gene-expression plasmids which contain (an) invertible promoter(s) and a multiple cloning site. We used either the plac promoter or the ptac-plac tandem promoters, the latter directing a more than fourfold increase in expression of the galK reporter gene in Escherichia coli host. All these plasmids were derived from the pNH7a **expression plasmid** of Podhajska et al. [Gene 40 (1985) 163-168]. Like pNH7a, these vectors have three novel properties: (i) in the 'OFF phase', the **promoter** is facing away from the gene to be expressed, (ii) the 'ON phase' is attained by the rapid and efficient inversion of the **promoter** mediated by the phage lambda Int product and the flanking **attP** and **attB** sites, which have a divergent orientation, and (iii) only a short heat pulse is required for the efficient inversion of the **promoter** and switching from the OFF to the ON phase. As for the pNH7 a vector, the present plasmids contain the nut-N transcriptional antitermination system, which permits efficient gene expression even if terminator(s) happen to be present between the promoter(s) and the expressed gene. The promoter inversion is rapid and over 95% efficient, as assayed by restriction analysis and galactokinase assay. Many genes could be conveniently cloned in the multiple cloning site, and then either kept totally silent or expressed in a rigidly controlled manner. Moreover, the pNH8, pNH16 and pNH18 plasmids, with already inverted promoters, could be used for expression of cloned genes, either in an unregulated manner or regulated by the lac repressor. They would be particularly useful for genes associated with terminators affecting their expression.

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L25 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 92268044 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1587805
TITLE: Modulation of active renin secretion by renin-binding protein (RnBP) in mouse pituitary AtT-20 cells transfected with human renin and RnBP cDNAs.
AUTHOR: Inoue H; Takahashi S; Miyake Y
CORPORATE SOURCE: Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka.
SOURCE: Journal of biochemistry, (1992 Mar) 111 (3) 407-12.
Journal code: 0376600. ISSN: 0021-924X.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920710
Last Updated on STN: 19970203
Entered Medline: 19920623

AB To investigate the role of renin-binding protein (RnBP) in renin metabolism, RnBP **expression plasmid**, which was constructed to express human RnBP under the control of mouse mammary tumor virus long terminal repeat, was transfected into mouse pituitary **AtT-20** cells together with the **expression plasmid** encoding human renin. The transfectant secreted prorenin and active renin, whereas RnBP was expressed only in the presence of dexamethasone and without secretion into the medium. The secretion of active renin was stimulated by forskolin, and the stimulation was repressed by dexamethasone. The secretion of prorenin, however, was insensitive to forskolin irrespective of the presence or absence of dexamethasone. Moreover, the forskolin-stimulated release of active renin was hardly repressed by dexamethasone in **AtT-20** cells transfected with the renin **expression plasmid** and a **selectable** plasmid pMAMneo. Coexistence of RnBP and renin mRNAs in human Wilms' tumor G-401 cells was shown by means of polymerase chain reaction of respective cDNAs from the cells. These results suggest that RnBP modulates the release of active renin in renin-producing cells.

L25 ANSWER 2 OF 4 MEDLINE on STN
ACCESSION NUMBER: 90194998 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2697983
TITLE: The molecular biology of human renin and its gene.
AUTHOR: Baxter J D; James M N; Chu W N; Duncan K; Haidar M A; Carilli C T; Reudelhuber T L
CORPORATE SOURCE: Metabolic Research Unit, University of California, San Francisco 94143.
CONTRACT NUMBER: 5 R01 HL 35706 (NHLBI)
SOURCE: Yale journal of biology and medicine, (1989 Sep-Oct) 62 (5) 493-501. Ref: 42
Journal code: 0417414. ISSN: 0044-0086.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19970203
Entered Medline: 19900424

AB The molecular biology of renin, prorenin, and the renin gene have been studied. A tissue-specific pattern of expression was found in rat and human tissues. In the human placenta, the transfected and endogenous renin promoters are active, and renin mRNA levels and transfected **promoter** activity are increased by a calcium ionophore plus cAMP. Cultured pituitary **AtT-20** cells transfected with a preprorenin **expression vector** mimic renal renin release by converting prorenin to renin and releasing renin in response to 8Br-cAMP.

Studies with mutant renin genes suggest that the body of renin directs renin to the regulated secretory pathway, and renin glycosylation affects its trafficking. Chinese hamster ovary cells were used to produce recombinant prorenin. Infused prorenin was not converted to renin in monkeys. Renin crystals were used to determine its three-dimensional structure. Renin resembles other aspartyl proteases in the active site and core, but it differs in other regions that probably explain renin's unique substrate specificity. Based on structural and mutational analysis, a model for human prorenin was built that suggests lysine -2 of the prosegment interacts with active site aspartate residues, and that the prosegment inactivation of renin is stabilized by binding of an amino terminal beta strand into a groove on renin.

L25 ANSWER 3 OF 4 MEDLINE on STN
ACCESSION NUMBER: 88330857 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2843521
TITLE: Expression of porcine cholecystokinin cDNA in a murine neuroendocrine cell line. Proteolytic processing, sulfation, and regulated secretion of cholecystokinin peptides.
AUTHOR: Lapps W; Eng J; Stern A S; Gubler U
CORPORATE SOURCE: Department of Molecular Genetics, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.
SOURCE: Journal of biological chemistry, (1988 Sep 15) 263 (26) 13456-62.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198810
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19881019

AB The cDNA for porcine preprocholecystokinin (pre-pro-CCK) was engineered for expression in mammalian cells under the control of the Rous sarcoma virus-long terminal repeat **promoter**. This **expression construct** was transfected into the murine anterior pituitary cell line, AtT-20. A stable cell line (AtT-20/CCK) was derived that expresses CCK mRNA indistinguishable from the CCK mRNA found in pig brain or gut. The AtT-20/CCK cells carry out proteolytic processing and sulfation reactions to generate authentic sulfated CCK8 from pro-CCK. The cells also store and secrete CCK-immunoreactive peptides. This secretion can be stimulated with corticotropin releasing factor, the natural secretagogue for anterior pituitary cells. In contrast, monkey kidney epithelial cells (COS cells), which are transiently transfected to express CCK, predominantly secrete nonsulfated pro-CCK into the medium. These studies show that a murine neuroendocrine cell line contains the complete processing machinery required to generate authentic porcine CCK8. The processing events include simultaneous proteolytic processing at one and two basic amino acid sites and sulfation of tyrosine residues. The cell line thus duplicates exactly the processing patterns found to occur in pig brain cortex.

L25 ANSWER 4 OF 4 MEDLINE on STN
ACCESSION NUMBER: 88007591 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3654635
TITLE: Transfected human neuropeptide Y cDNA expression in mouse pituitary cells. Inducible high expression, peptide characterization, and secretion.
AUTHOR: Dickerson I M; Dixon J E; Mains R E
CORPORATE SOURCE: Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
CONTRACT NUMBER: DA-00097 (NIDA)
DK-18849 (NIDDK)
DK-32948 (NIDDK)
SOURCE: Journal of biological chemistry, (1987 Oct 5) 262 (28) 13646-53.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198711
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871109

AB An **expression vector** was constructed that placed the cDNA for human neuropeptide Y (NPY) under the control of the mouse metallothionein **promoter** and was used to transfect the **AtT-20** mouse anterior pituitary corticotrope cell line. **AtT-20** cells normally process the pro-ACTH/endorphin precursor but do not produce detectable levels of NPY. The resulting **AtT-20/NPY** cell line (Mt.NPY1a) was used to study the ability of the corticotrope cells to synthesize, process, and secrete the foreign proNPY-related peptide products. The stable cell line created contains approximately 40 copies of proNPY cDNA per cell. NPY mRNA levels and proNPY synthesis were increased at least 35-fold when maximally induced with cadmium; proNPY synthesis was also induced by glucocorticoids. Upon induction the NPY secretion rate was equimolar to that of the endogenous peptides. ProNPY, NPY, and the COOH-terminal peptide produced by this cell line had molecular weight and amino acid-labeling pattern predicted from cDNA sequence data and from previous isolation of NPY-related molecules from NPY-producing cells. The structures of secreted proNPY, NPY, and COOH-terminal peptide, as well as determination of the site of proteolytic cleavage between NPY and the COOH-terminal peptide, were determined by tryptic mapping and Edman degradation of secreted biosynthetically labeled peptide products. The proNPY molecule appears to be processed in the same pathway responsible for cleavage of the endogenous pro-ACTH/endorphin precursor. Secretion of proNPY-derived peptides paralleled secretion of endogenous pro-ACTH/endorphin-derived products, under both basal and stimulated conditions. With induction proNPY expression there is a dose-dependent inhibition of both proNPY and pro-ACTH/endorphin proteolytic processing.

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